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Total word count for the main body of the text (Introduction, Materials and Methods, Results, Discussion, and Acknowledgements): 5,944

Word counts for each section

1. **Introduction**: 1,238 words
2. **Properties of initial sex-determining mutations**: 2,259 words
3. **Has close linkage evolved in response to a sex-determining locus, or did a sex-determining mutation arise in a pre-existing non-recombining region?** 1,432 words
4. **Are single-gene systems derived?** 840 words
5. **The ages of plant and fish sex chromosomes**: 280 words
6. **Conclusions**: 532 words

Number of figures (indicating which figures should be published in colour): 3 (all with colours)

Number of tables: 0

There is no supporting information

Abstract (196 words)

A major reason for studying plant sex chromosomes is that they may often be “young” systems. There is considerable evidence for the independent evolution of separate sexes within plant families or genera, in some cases showing that the maximum possible time during which their sex-determining genes have existed must be much shorter than those of several animal taxa. Consequently, their sex -linked regions could either have evolved soon after genetic sex determination arose, or considerably later. Plants therefore include species with both young and old systems. I review several questions about the evolution of sex-determining systems and sex chromosomes that require studies of young systems, including (i) the kinds of mutations involved in the transition to unisexual reproduction from hermaphroditism or monoecy (a form of functional hermaphroditism), (ii) the times when they arose, and (iii) the extent to which the properties of sex-linked regions of genomes reflect responses to new selective situations created by the presence of a sex-determining locus. I also evaluate which questions are best studied in plants, versus other suitable candidate organisms. Studies of young plant systems can help understand general evolutionary processes that are shared with the sex chromosomes of other organisms.

Keywords in alphabetical order: Flower development, gene duplications, sex chromosomes, sex linkage, turnover events, Y chromosomes

1. Introduction

Although plant sex chromosomes are often stated to be "young", the reasons for studying young systems are less often explained. I here examine several questions concerning the early evolution of sex-linked regions and sex chromosomes, and discuss the organisms in which they can be studied, to clarify the advantages of studying sex chromosomes of plants. As the box outlines, sex chromosomes, broadly defined, are surprisingly varied, and, although their ages can only be defined relatively to those of such chromosome systems in other organisms, and not in absolute terms, both young and old systems are present among plants, which therefore include examples of most of the range of different sex chromosome types possible.

I concentrate on the initiation and evolution of separate sexes (called gonochory by zoologists, and dioecy here, following botanical usage), and genetic sex-determination. I also consider the evolution of genome regions that carry sex-determining genes. In species in which males are the heterozygous sex (male heterogamety), the fully sex-linked regions, with no crossing over between the regions determining the two sexes are called Y- and X-linked, and males are denoted by XY, whereas in species with female heterogamety, females are heterozygous for Z- and W-linked regions and males are ZZ. Sex chromosomes often also include regions that undergo genetic recombination, called "pseudo-autosomal" regions, or "PARs". Even if the fully sex-linked region is very small, for example, a single gene, and most of it is a partially sex-linked PAR, the chromosome carrying the Y- (or W-) linked region may still be called a sex chromosome. It is important to understand that such a chromosome will often not have the characteristics of the most familiar ancient sex chromosomes, such as those of mammals or Drosophila or birds, with major gene losses from the Y or W chromosomes (genetic degeneration), causing hemizygosity in one sex (summarised in the box).

In order to concentrate on the evolution of young sex chromosomes, I will not discuss the interesting evolutionary consequences that follow from the evolutionary processes affecting fully sex-linked regions in detail, including loss of functions of genes, or deletion of Y- or W-linked genes, known as "genetic degeneration". These have been reviewed recently (e.g. Bachtrog, 2012), and have been detected in plants (Hough *et al.*, 2014), and are summarised in the box. An interesting question, reviewed recently (Lorenzo *et al.*, 2018), concerns the

relative importance of deleterious mutations becoming fixed in the Y (or W) chromosome population, versus accumulation of repetitive sequences, which are predicted to accumulate in non-recombining regions (Charlesworth *et al.*, 1994). Transposable elements are a major proportion of repetitive sequences in plant genomes, and their accumulation can account for low gene densities in sex-linked regions (Wang *et al.*, 2012). They may also contribute to genetic degeneration, as their transposition can cause mutations, including deletions and chromosome rearrangements. Genome processes that evolved to suppress their transposition can also suppress expression of nearby genes (Hollister & Gaut, 2009), perhaps initiating Y chromosome degeneration (Zhou & Bachtrog, 2012).

Studying young systems requires evidence supporting their recent evolutionary origins. One definition of young systems is that they have not yet undergone major degeneration, for example the Y-linked region in *Asparagus officinalis* (Harkess *et al.*, 2017b), or the guppy, a fish whose Y chromosome appears to carry all genes found on the X (Bergero *et al.*, 2019). However, these are rough measures of age, as degeneration rates depend on many organism-specific properties (see box). Estimating the rate of degeneration requires having reliable age estimates, including ages relative to those of other sex chromosome systems, as outlined in the box. I shall not discuss approaches for estimating the ages of sex chromosomes in detail, but note here that each of the two main approaches has advantages and disadvantages. Phylogenetic studies can identify clades with genetic sex determination, and the clade's absolute age in generations or years can be estimated using divergence for synonymous or silent sites in DNA sequences, preferably using multiple nuclear gene orthologues in different species (such divergence can also be used to assess relative ages, ideally taking account of substitution rate differences). This might not identify the origin of the system correctly, because it is difficult to distinguish between multiple recent evolutionary changes, and the existence of an ancestral system that has reverted several times and lost dioecy. Figure 1 illustrates different evolutionary histories for a set of species with some dioecious and some hermaphrodite taxa, starting from an ancestrally functionally hermaphrodite state (including monoecy in a plant).

Alternatively, estimates can be based on divergence between sequences of genes in the sex-linked region (if one exists). Divergence between X and Y sequences of a fully sex-linked gene estimates the time when this "male-specific Y", or MSY, region stopped recombining

with its X counterpart (and similarly for W-Z divergence). However, different sex-linked genes may stop recombining at different times in the system's evolution. So-called "evolutionary strata", each representing a chromosome region that stopped recombining at a given time, and differing in divergence from other strata, have been found in mammals (Lahn & Page, 1999), several plants (Bergero *et al.*, 2007; Wang *et al.*, 2012), and a fish, the threespine stickleback (Schultheiß *et al.*, 2015; White *et al.*, 2015).

In addition to helping assign time of origin of sex chromosome systems, strata can help identify the sex-determining locus, as this is expected to lie within the oldest sex-linked region. The discovery of such strata is also important because their presence on sex chromosomes tells us that recombination stopped in events subsequent to the initial establishment of a sex-determining region on the chromosome, suggesting that recombination suppression is an evolved response to sex-specific adaptation (see the box and the sections 2 and 3 below).

However, the existence of strata is still difficult to test, as exemplified by uncertainties in sex chromosomes of fish such as the Atlantic cod (Kirubakaran *et al.*, 2019) and some cichlid species (Gammerdinger & Kocher, 2018a). Strata are most likely to be detectable in moderately young sex chromosomes. Detecting strata requires a reliable physical map of the chromosome carrying the sex-determining genes. The X chromosome genetic map is often used as a proxy for the physical arrangement of the sequences used. Sex-linkage can be identified if variants with X-linked inheritance are discovered, or genome regions are found with lower representation in one sex than the other, indicating hemizygosity in males in XY systems (or females in ZW systems). Chromosome level genome assembly methods are now becoming reliable enough to avoid the need for laborious genetic mapping and directly obtain physical arrangements for both X and Y chromosomes. However, several difficulties in detecting strata remain. First, Y-X divergence estimates require Y-linked sequences. Genes may be very rare in ancient systems' degenerated old strata (see Box). Assigning a time of origin of the sex chromosome requires divergence estimates for sequences from the region that stopped recombining longest ago; unless the oldest stratum is included, the age will be under-estimated. It may also be impossible to estimate enough sequence divergence values to detect clear differences between different sex chromosome regions, given these estimates' high variances. Moreover, sequence divergence between the

two sex chromosomes may be saturated in old strata, further obscuring any differences defining strata. The small number of species where sex chromosome strata have been identified should increase as sequence information become available from more species, especially as knowledge of systems ages accumulates, and suitable ages can be targeted for genome sequencing.

2. Properties of initial sex-determining mutations

One reason for studying young sex chromosomes is to discover the genes that may have been involved in the change from an ancestral functionally hermaphrodite state, including (in plants) monoecy, to two separate sexes. Discovering the genes involved will reveal whether the mutations actually involved have the properties assumed in these models. Different routes to dioecy can be imagined, involving at least two initial mutations, one to produce each sex (Charlesworth, B & Charlesworth, D, 1978). Because transitions from functional hermaphroditism to dioecy have occurred many times in flowering plants (Charlesworth, 1985; Renner, 2014), they are particularly good for studying which of these possible routes has, in fact, been involved.

A mutational model that seems plausible for natural transitions to dioecy from hermaphroditism (rather than requiring intervention by plant geneticists, see below), seems likely most often to involve a major male-sterility mutation, creating females and producing a gynodioecious population, followed by one or more mutations converting the hermaphrodite individuals to more male-like forms (Westergaard, 1958). This genetic pathway can apply to an ancestor with hermaphrodite flowers or a monoecious ancestor. In the simplest case, the second mutation converts (functional) hermaphrodites to full males, with complete loss of female functions. Males created by a female-suppressor mutation can be advantageous once females are established in a population, but female fitness is inevitably decreased, and the mutation is sexually antagonistic. Both loci will become polymorphic in the population (since neither male- nor female-sterility can become fixed), generating selection for closer linkage between the two mutations (see next section).

Sterility through loss-of-function mutations and through suppressive mutations

Genetic data show that mutations in distinct genes are involved in several plants where relevant information can be obtained. Examples where the ancestor was probably monoecious include *Ecballium* (Cucurbitaceae) and spinach (Amaranthaceae), *Sagittaria latifolia* (Alismataceae), and the date palm genus *Phoenix* (Westergaard, 1958; Dorken & Barrett, 2004; Nadot *et al.*, 2016; Torres *et al.*, 2018; Wadlington & Ming, 2018). There is also good evidence for the involvement of two distinct genes in at least two species where dioecy appears to have evolved from hermaphroditism, *Silene latifolia*, based on the phenotypic effects of deletions of Y-linked regions (Westergaard, 1958; Kazama *et al.*, 2016), and *Asparagus officinalis*, in which genome sequencing yielded candidate male-sterility and female-suppressing genes (Harkess *et al.*, 2017b; Harkess *et al.*, 2018). Many loci in plants can generate loss-of-function mutations causing male sterility (Ohnishi, 1985; Timofejeva *et al.*, 2013), and such mutations are generally recessive, as assumed in the model above. The resulting female homozygotes could have high-fitness if inbreeding depression disfavours individuals with both male and female functions (Charlesworth, B & Charlesworth, D, 1978). It is important to evaluate the evidence for the two gene model in as many plants as possible, because, as illustrated in Figure 2, it predicts that recombinant combinations of alleles will be disfavoured, favouring close linkage and subsequent suppression of recombination, between the two genes involved (Charlesworth, B & Charlesworth, D, 1978). This model therefore potentially explains the repeated loss of recombination that appears to have evolved repeatedly between members of sex chromosome pairs (Bull, 1983). If the two locus hypothesis can be excluded in an organism with a sex-linked region where empirical testing is possible, other evolutionary causes must be sought for the evolution of its sex-linked region, as will be discussed below. It should be noted, however, that it is currently unclear how many cases of the evolution of dioecy have been followed by recombination suppression events (see section 3 below).

Trade-offs during reproduction and possible evolution of dioecy by two mutations in a single primary sex-determining gene

Mutations in separate genes, need not necessarily always be involved. An important assumption of the model just outlined is that the second mutation is unlikely to be a simple sterility mutation. Population genetic analysis shows that avoidance of inbreeding

depression cannot be the sole selective force favouring males, so that considerably increased male fertility, relative to that of the ancestral hermaphrodites, is required to compensate for loss of female fertility (Charlesworth, B & Charlesworth, D, 1978). Furthermore, while femaleness is often recessive (consistent with a loss-of-function mutation creating XX females), the mutation creating males must be expressed in heterozygous (XY) individuals. Partial dominance of the female suppressor suggests that it might control resource allocations during flower development. Re-allocating the functional hermaphrodites' resources towards greater investment in male functions can be advantageous in gynodioecious populations.

This idea of a trade-off between male versus female functions further suggests that a single gene could, in principle, undergo two mutations to produce both sexes from an ancestral functionally hermaphrodite state: mutations reducing male functioning should increase female function, and *vice versa*, potentially producing a single sex-determining gene. A gene with such developmental properties has indeed been discovered in a liverwort whose gametophytes are either male or female, *Marchantia polymorpha* (Hisanaga *et al.*, 2019). It appears to encode an MYB class transcription factor protein whose loss (or loss-of-function), converts female gametophytes to males that produce non-functional sperm (they presumably lack sex-linked genes essential for producing functional sperm). An anti-sense transcript expressed from the same gene acts to repress the transcription factor, and its loss-of-function mutations convert male gametophytes into non-functional female ones. A function in directing development into male or female pathways is therefore likely. However, this gene is not sex-linked in *M. polymorpha*, and is therefore not the primary sex-determining gene, but must act downstream of the primary control.

A situation in which partial (rather than complete) loss of male or female functions is plausible involves male-female flower ratios in monoecious plants, although mutations reducing the proportion of male flowers are less likely to be advantageous, compared with sterility mutations, as the rate of self-fertilisation might often be only slightly reduced (Charlesworth, D & Charlesworth, B, 1978). However, the assumptions of this theoretical model have been little tested, and more studies of the relationship between male/female flower numbers and selfing rates in monoecious plants would be helpful. Furthermore, if the argument above is correct that such mutations should generally show intermediate

dominance, the recessivity of one sex observed in many plants and animals is puzzling: even in plant systems that are probably young, male heterogamety, with femaleness being recessive, is common (Westergaard, 1958).

In a species that has recently evolved dioecy, new, secondary mutations improving the functioning of one sex may quickly become incorporated in genome regions closely linked to the sex-determining gene(s), and will be hard to distinguish from the initial mutations. The genome region closely linked to the sex-determining locus of *Asparagus officinalis* includes several genes likely to affect development of flower parts (Harkess *et al.*, 2017a; Murase *et al.*, 2017). Genes with sex differences in expression are also found unexpectedly often in the 14.5 Mb apparently fully sex-linked region of the *Mercurialis annua* genome (Veltsos *et al.*, 2018) and a 2 Mb pericentromeric region on the *Salix purpurea* chromosome, 15, carrying this species' ZW system's sex-determining locus (Carlson *et al.*, 2017). Gender-specific expression is, of course, also found for genes unlinked to such regions (West & Golenberg, 2018), reflecting either effects in development downstream of the sex-determination step, or adaptations to the separate sexed condition. The extent to which newly dioecious plants adapt to unisexual functioning is not yet clear, but gene expression changes can suggest possible adaptive changes. For example, female *Silene vulgaris* (a gynodioecious species in the Caryophyllaceae) have been used to estimate the direct effects of loss of androecium on gene expression in flower buds, and show that expression changes in *S. latifolia* females are larger, consistent with evolved changes in this dioecious species (Zemp *et al.*, 2018).

Other mutation types that could generate dioecy

Hypotheses for routes to the evolution of dioecy are not restricted to the mutation types outlined above (the two gene model with male-sterility followed by a female suppressor was proposed in order to understand why recombination often becomes suppressed in genome regions carrying sex-determining loci). In persimmon (Ebenaceae, a dioecious system whose age is intermediate between those of many fish discussed below and those of ancient sex chromosomes in mammals and birds) two mutations were involved, with properties differing from those in the model outlined above, as illustrated in Figure 3; the figure is adapted from the model proposed for this system (Akagi *et al.*, 2014) to show a plausible evolutionary pathway. As in the loss-of-function scenario above, a mutation in a

gene named *MeGI* probably created females first. In the ancestral hermaphrodite, this gene promotes male function, perhaps by permitting expression of another gene necessary for some male function. The mutant product suppresses the male function gene's expression, producing females; this would not be expected to be fully recessive, but should act even in heterozygotes. Experiments expressing MeGI in the hermaphroditic plant *Arabidopsis thaliana* suggest that androecium sterilization leads to increased female functions through a trade-off effect, as Darwin (Darwin, 1877) suggested, rather than MeGI having a second action directly promoting gynoecium development (Akagi *et al.*, 2014). Like any male-sterility mutation, this mutation might establish a polymorphism for females in the population (provided that deleterious pleiotropic effects do not prevent this).

A non-mutant *MeGI* copy duplicated onto a different chromosome, named "*OGI*", then arises and dominantly blocks the expression of the *MeGI* gene, changing the ancestral bisexuals into males (Akagi *et al.*, 2014). In the presence of the duplication, *MeGI's* wild-type male-promoting action is assumed to be stronger than that of the ancestral allele, so that female functions are suppressed through the trade-off effect proposed above. As in the loss-of-function mutational model outlined above, males could have become advantageous once females became common in the population.

In this model, the *MeGI* gene polymorphism can be lost, as all members of the population can carry the *MeGI* mutation that causes femaleness, because this is active only in females (in which its product blocks male functions). The resulting population would then be polymorphic only for the *OGI* duplicate, and the chromosome with this gene would behave as a Y-linked region with a single gene controlling whether the individual is male or female (even though the developmental system involved of course includes several other genes, and the evolution of the present system involved at least two mutations).

Note that the term "Y-linked region" here does not imply that suppressed recombination has evolved, but merely that a sex-determining locus is present. However, it is worth noting that the insertion of the OGI duplicate into an ancestral autosome (indicated as a black line in Figure 3), creates a region that is hemizygous in males, and might prevent pairing with its homologue. If so, the surrounding genome region might include several co-segregating genes with no involvement in sex-determination, resembling a miniature version of the situation in sex chromosomes that have evolved suppressed recombination. In the

299 persimmon, an approximately one megabase region shows Y linkage (Akagi *et al.*, 2014).
300 Suppressed recombination near such an inserted male-determining gene is not an evolved
301 response to the presence of a sex-determining gene, and certainly not to a situation with
302 two partially linked polymorphic genes selecting against recombinant haplotypes.

303 This complicated model is just one possibility for systems in which a single gene controls
304 male versus female development. It was long thought that "originally there must have been
305 just one gene that played the critical role in the sex-deciding process" (1932), and separate
306 sexes can indeed be produced in the normally monoecious maize, by combining recessive
307 mutations in two maize genes (reviewed by Jones 1934). Homozygotes for the silkless
308 mutation are males, due to failure of female flowers, but homozygosity for the tasselseed
309 mutation causes male (terminal) inflorescences to form flowers with female function. In a
310 population of silkless homozygotes, gender can therefore be controlled by segregation of
311 alleles of the single tasselseed gene, with males heterozygous for the wild-type allele,
312 resembling a dominant maleness factor. Unlike the persimmon case, however, the wild-type
313 silkless allele must be deliberately excluded, because the females would be eliminated
314 without artificial intervention, due to low fertility.

315

316 *Predicted lack of pleiotropy*

317 As explained above, sterility mutations involved in the natural evolution of separate sexes
318 must confer some fitness advantage: females avoid producing inbred offspring, and males
319 can optimize their resource allocation in response to the presence of females. There is
320 evidence for both these effects in plants evolving dioecy, for example in *Wurmbea species*
321 (Liliaceae) (Barrett, 1992; Ramsey *et al.*, 2006). Also, it is obvious that mutations with
322 detrimental pleiotropic effects on other tissues should rarely be involved in the evolution of
323 dioecy: sterility mutations in plant genes that act in the anthers or their development will be
324 eliminated unless female functions are unaffected (or enhanced), and vice versa. With
325 modern developmental approaches and understanding of flower development, it may be
326 possible to test this prediction. For instance, transgenic experiments in hermaphrodite
327 species could be used to test genes whose loss of function causes male or female sterility, to
328 discover which genes have such side effects, and whether candidate primary sex-
329 determining genes identified on actual sex chromosomes do not. Hermaphrodites closely

related to dioecious species could also be used to test for the expected resource re-allocation. Given the shared flower developmental systems of angiosperms, even unrelated species could potentially be used in testing for pleiotropy or its absence. An experiment expressing the persimmon *MeGI* feminization factor in the distantly related hermaphrodite plant *Nicotiana tabacum*, using *N. tabacum* promoters, indeed found few pleiotropic effects, though high expression of *MeGI* in *Arabidopsis thaliana* led to sterile androecia (as expected), and normal carpels, but also stunted growth (Akagi *et al.*, 2014).

3. Has close linkage evolved in response to a sex-determining locus, or did a sex-determining mutation arise in a pre-existing non-recombining region?

Non-recombining completely sex-linked genome regions could arise in several ways. Models of the evolution of dioecy involving mutations in separate genes, and interacting in a manner that creates a disadvantage to recombinants (which, as outlined above, is not always the case), select for closer linkage between the two mutations (Bull, 1983). This could sometimes create sex-linked regions large enough to include many genes. In contrast, as mentioned above, an inserted male-determining gene could directly create a small sex-linked region.

Single gene systems

Selection favouring closer linkage between two mutations occurs only if the mutations initially recombine; if they are within the same gene, they will be closely linked from the time they originated, again forming a small sex-linked region. Single gene systems have been discovered in several fish, including fugu (Kamiya *et al.*, 2012) and Atlantic salmon (Kirubakaran *et al.*, 2019), and could be involved in plants with homomorphic sex chromosomes, which appear to be common among angiosperms (Westergaard, 1958). A single gene system can evolve via two mutations, one of which becomes fixed in the species (as appears to have happened in the persimmon, see above). In the next section, I explain how such systems could evolve by duplications and in other ways involving single genes taking over the sex-determining function. Before doing so, I note that extensive fully sex-linked regions can arise if new sex-determining genes evolve in genome regions that lack recombination.

Evolution of a new sex-determining gene in a non-recombining genome region

Another situation in which existence of a fully sex-linked region does not imply selection driven by the presence of sex-determining genes is when such genes evolve within an already non-recombining genome region. Current information about the relationship between physical and genetic maps is scanty, as both well-assembled genome sequences and genetic maps are required. However, it is now possible to get relevant data if a chromosome level assembly of a species' genome sequence can be obtained and combined with genetic mapping of families. Clearly, this is still difficult, as large genomes with substantial repeat content, such as those of many organisms, are difficult to assemble and will currently inevitably include some errors. Obtaining families from species of interest for sex chromosome evolution is also often difficult. Among angiosperms, dioecious species tend to be trees or shrubs (Darwin, 1877; Renner, 2014), rather than species with short generation times suitable for genetic studies. Moreover, many such plants have large genome sizes that are difficult to assemble, and some have undergone whole genome duplications (Pellicer *et al.*, 2018).

Nevertheless, many dioecious plants have non-dioecious relatives that may allow comparisons to test whether a sex-determining region evolved its non-recombining state in the newly dioecious lineage. Currently, few comparisons of recombination patterns have been published, and it is rarely known whether recombination changed after a species evolved separate sexes, or after a new male-determining factor has appeared on a chromosome. In the ninespine stickleback, a maleness factor has evolved in a region that rarely recombines (due to a polymorphic inversion); this case is an example where the lack of recombination pre-dates its sex-determining role (Natri *et al.*, 2019).

It may be common for a sex-determining locus to evolve within a pericentric genome region in which recombination is rare. Genetic maps in animals and plants, pooled for male and female meiosis, are revealing that (for evolutionary reasons that are not well understood) such regions can represent large proportions of chromosomes' physical sizes, including in maize (Kianian *et al.*, 2018) and *A. thaliana* (Giraut *et al.*, 2011). In barley (*Hordeum vulgare*), crossovers are localised to small end regions of the physically huge chromosomes, based on genotyping single pollen grains (Dreissig *et al.*, 2015; Dreissig *et al.*, 2017). Although the central regions have low gene density, some genes are present and sex-

391 determining loci could evolve in these regions. In *Petunia*, the genes controlling self-
392 incompatibility types, which requires maintaining suitable combinations of alleles of at least
393 two genes (similar to the two-gene model for the evolution of separate sexes), evolved in a
394 pericentric region that recombines rarely (TenHoopen *et al.*, 1998). Sex-linkage from the
395 moment a sex-determining locus arises, for this reason, has been proposed for the oldest Y-
396 linked region of papaya (*Carica papaya*) (Zhang *et al.*, 2008). The sex-linked regions in
397 spinach species (a genus in which dioecy probably evolved from a monoecious ancestral
398 state) may also have arisen in this way. The homomorphic XY chromosome pair exhibits
399 extensive synteny with sugar beet chromosomes 4 and 9, and protein-coding genes closely
400 linked to the male-determining locus in spinach are located in the putative pericentromeric
401 and centromeric regions of these *Beta vulgaris* chromosomes (Takahata *et al.*, 2016), while
402 much of the pair recombines. High-density mapping identified an 18 Mb region of linkage
403 group 4, with 143 genes, as fully sex linked (Qian *et al.*, 2017); its location is consistent with
404 a pericentromeric region, as is the low gene density of the region (Kudoh *et al.*, 2018). This
405 may be a young system, as a rare viable YY homozygote has been observed, suggesting that
406 there has not been enough time for loss of essential genes from the Y (Wadlington & Ming,
407 2018). Interestingly, the homologous chromosome (carrying sequences that show sex-
408 linkage in different *Spinacia* species) is heteromorphic in one species, probably due to a
409 pericentric inversion that changed the arm ratio by altering the centromeric position (Fujito
410 *et al.*, 2015). It is not yet known whether this event expanded a fully sex-linked region, or, if
411 so, whether the expansion was favoured by the appearance of a mutation affecting sexual
412 dimorphism. However, intriguingly, inflorescence dimorphism is more pronounced in the
413 species with the expanded MSY, and mutations in a gene a few centiMorgans away lead to a
414 highly staminate monoecious phenotype (Onodera *et al.*, 2011).

415 Similarly, if recombination occurs only in one sex, any chromosome acquiring a sex-
416 determining factor will immediately become sex-linked. I am not aware of any plant with
417 such extreme sexual dimorphism in crossing over, but more analyses are needed of male
418 and female meiosis separately, in species with separate sexes. Highly sexually dimorphic
419 recombination patterns have recently been found in fish, for example a salmonid
420 (Sutherland *et al.*, 2017). In another fish, the guppy (*Poecilia reticulata*), cytogenetic
421 visualisation of crossovers in male meiosis suggests localisation near the tips of all the

422 acrocentric chromosomes (Lisachov *et al.*, 2015). Genetic mapping results (Bergero *et al.*,
423 2018) suggest that this sex difference in crossing over accounts for the large regions of the
424 chromosome carrying the sex-determining locus that do not recombine in male meiosis.
425 Genetic maps of related species should help distinguish between (i) a non-recombining state
426 pre-dating the present sex-determining locus evolved, versus (ii) selection against X-Y
427 recombination after this locus appeared on chromosome 12, leading to evolution of
428 genome-wide chiasma localisation in male meiosis.

429 Why recombination patterns or rates often differ between the sexes remains unresolved.
430 Hypotheses include differences between male and female meiosis (which can account for
431 differences observed in hermaphrodite species), or selection that is related to sex-linked
432 regions (Lenormand, 2003; Lenormand & Dutheil, 2005). The genetic control of localisation
433 patterns is also not currently clearly understood in any species, though genetic differences
434 in genomic patterns of recombination events, as well as rates, have been documented.,
435 including a difference in female meiosis between two closely related *Drosophila* species
436 (Brand *et al.*, 2018); males of almost all *Drosophila* species lack crossing over.

437

438 *Gene duplications in fish and plant sex determination*

439 Non-recombining regions may also arise through insertion of a duplicate gene copy into a
440 chromosome (see above). An insertion/deletion difference between the two alleles at the
441 distyly locus in plants in the genus *Primula* (Huu *et al.*, 2016; Li *et al.*, 2016), may have
442 prevented recombination between the two, and the asparagus sex-determining region
443 appears to lack recombination for the same reason (Harkess *et al.*, 2017b; Harkess *et al.*,
444 2018).

445 In a fish, the medaka (*Oryzias latipes*), the chromosome carrying the sex-determining locus
446 is cytologically homomorphic, but has an insertion of a duplicate of a member of a gene
447 family with involvement in male-determination in other species; this initial 43-kb has
448 subsequently accumulated 137 kb of repetitive sequences (Kondo *et al.*, 2009).
449 Recombination is suppressed near the sex determining region, presumably due to the
450 absence of a homologous sequence with which to pair, while the rest of the chromosome
451 recombines and appears to be pseudo-autosomal (Kondo *et al.*, 2001); a small inserted

region creates hemizygosity that may prevent recombination in the region, and in flanking regions that also cannot pair. Overall, however, this species' total genetic map lengths are similar in both sexes (Kimura *et al.*, 2006).

4. Are single-gene systems derived?

As described above, the genes involved in the initial evolution of separate sexes sometimes have properties that can lead to establishment of two-gene polymorphisms and will generate selection for closer linkage. However, if single gene systems prove to be common, an important question is whether they represent the loci involved in the initial evolution of separate sexes, or a derived state that has replaced an ancestral system. In either case, the sex chromosomes evolve from autosomes, but *de novo* evolution of sex-determining genes in an ancestral hermaphrodite should be distinguished from the appearance of a new sex-determining locus in a lineage that had already evolved separate sexes.

Changes of the second kind are well documented in several organisms, particularly in fish. Two types of event are possible. First, a new gene may take control of the sex determination system (bottom part of Figure 2). This must involve a gene in the system that controls development of the sex structures (Wilkins, 1995), or interacts with such a gene, for example in the rainbow trout (Bertho *et al.*, 2018). Many examples are known in fish where the sex-determining loci of related species are on different chromosomes. Examples are known in the genus *Oryzias* (Myosho *et al.*, 2015), and among cichlids, in which, among only 14 species that evolved in the past approximately 45 MY, 11 different chromosomes carry the sex-determining locus (Gammerdinger & Kocher, 2018a). Sometimes the new region carrying the locus is physically small, and includes no candidate sex-determining genes shared between the species. A change to a new location on the same chromosome is less easily detectable, but fine-scale genetic mapping has revealed an example in the pufferfish genus Takifugu (Ieda *et al.*, 2018). Transitions are known both from XY to ZW systems, and vice versa (Gammerdinger & Kocher, 2018a).

It is difficult to distinguish between "take-over" events, versus movement of an existing sex-determining gene to a new genome location. Both types of movement seem to have occurred in house flies (Meisel *et al.*, 2017). Plants with young sex chromosomes may also have undergone such events, and may be excellent species for studying them and

483 understanding the situations that promote such changes. The sex-determining factors in
484 several closely related species of section *Otites* in the genus *Silene* map to different
485 chromosomes, all non-homologous with the XY pair in *S. latifolia* and its close relatives
486 (Balounova *et al.*, 2019). Both male and female heterogamety are found among these
487 species, yet the sex chromosomes in this section evolved very recently, and the non-
488 recombining region of the *S. otites* W-chromosome carries few genes. One hypothesis,
489 involving mutational load (Blaser *et al.*, 2013) favours a new (or re-located) sex-determining
490 locus if a sex-linked region is old enough to have undergone genetic degeneration, but has
491 not yet evolved dosage compensation. This probably does not explain the changed sex-
492 determining regions in section *Otites*, but it may explain changes in other taxa that
493 repeatedly evolved new sex-determining loci. Other classes of models to account for such
494 "turnover" events are reviewed by Blaser *et al.* (reviewed in Blaser *et al.*, 2013). They
495 include sex ratio selection, including responses to sex ratio distortion due to meiotic drive
496 (Scott *et al.*, 2018), a polymorphism for sexually antagonistic alleles making a new sex-
497 determining mutation advantageous if it arises in a closely linked region (vanDoorn &
498 Kirkpatrick, 2007), escape from aneuploidy caused by chromosomal inversions associated
499 with non-recombining sex-linked regions (Blackmon & Brandvain, 2017). Selectively neutral
500 changes can also lead to changed sex determination in a population, because dominant
501 alleles are predicted to replace ones with lesser dominance (Veller *et al.*, 2017; Saunders *et*
502 *al.*, 2018).

503 No case is yet known of a plant with a different sex-determining locus replacing an
504 established one, but movements of a pre-existing sex-determining factor have been
505 uncovered in octoploid species in the plant genus *Fragaria* (Tennessen *et al.*, 2018). Fine-
506 scale genetic mapping, combined with sequencing, identified similar genes and their
507 flanking sequences in different locations at which sex-determining factors map. This plant
508 seems to have undergone transposition events very similar to those of the region containing
509 the ~ 4kb sex-determining locus that is inferred to have moved between different
510 chromosomes in Atlantic salmon, and its close relatives (Woram *et al.*, 2003; Faber-
511 Hammond *et al.*, 2015; Lubieniecki *et al.*, 2015); recent results are reviewed in Kijas *et al.*
512 (2018). The insertions may prevent recombination in the small Fragaria genome regions
513 affected (Tennessen *et al.*, 2018), as in the case of the insertion in the medaka fish.

514 However, extensive degenerated non-recombining regions are not created, and therefore
515 the movements probably again did not occur due to selection created by mutational load. It
516 is currently unknown how such transpositions occur; they could involve recombination
517 between homeologous chromosomes of these polyploid species, or movements by
518 transposable elements (TEs), some of which have the potential to move host genes, for
519 example in maize (Morgante *et al.*, 2005; Dong *et al.*, 2011). TEs have contributed to
520 duplicative movements of nucleotide-binding site (NBS)-encoding disease resistance (NBS-R)
521 in the grapevine genome, and defence genes in Brassicaceae (van-den-Bergh *et al.*, 2016).
522 Multiple gene fragments are often found within single maize helitrons, indicating that
523 several sequential transpositions have happened during their history, as proposed in
524 *Fragaria*.

525

526 **5. The ages of plant and fish sex chromosomes**

527 Turnover events of either of the two kinds just described can create single gene sex-
528 determining loci, creating young sex-linked systems in species with a different ancestral
529 state. Another way in which new sex-linked genome regions can evolve, potentially creating
530 extensive, multi-gene sex-linked regions if new sex-determining loci evolve within non-
531 recombining regions. This could be the situation in *Rumex*, where the sex chromosomes of
532 two species groups are non-homologous, and one appears to be old (with a highly
533 heteromomorphic XY pair carrying a high proportion of genes with high sequence
534 divergence), and the other with much less XY differentiation, and therefore probably
535 recently evolved (Crowson *et al.*, 2017). It will be important to understand whether, in
536 plants, turnover events replacing older systems by new ones are as common as the loss of
537 dioecy and its re-evolution.

538 Determining ages of systems will be important in relation to the question of whether single
539 gene systems reflect *de novo* evolution involving two mutations in a single gene. If dioecy is
540 shared by several related species, it could initially have evolved long ago, but successive
541 turnovers could have occurred, persisting too briefly to evolve large non-recombining
542 regions and chromosome heteromorphism (Rodrigues *et al.*, 2018). Overall, it is currently
543 unknown what determines why a large fully sex-linked region sometimes evolves, or why a
544 turnover event sometimes occurs before this has occurred. Plants with homomorphic sex

chromosomes could be valuable for such studies, as closely related outgroup species may be available to infer changes.

6. Conclusions

I have argued here that some of the properties of sex chromosomes may not have evolved in response to the presence of a sex-determining locus. I have concentrated on the concept that a lack of recombination need not reflect suppression of crossing over between two genes involved in sex-determination, or one sex-determining gene and a gene that mutates to an allele that benefits only one sex and is disadvantageous in the other sex (i.e. is sexually antagonistic). Young systems may be ideal for testing whether recombination rates have changed, similarly to testing whether the molecular evolution of the lineages leading to extant Y (or W) chromosomes indicates genetic degeneration of sequences carried on the ancestral chromosome that evolved into the sex chromosome pair (and which are still present on the X or Z).

A similar question relates to sex chromosome evolution after an extensive sex-linked region has evolved and has undergone genetic degeneration. In the Y-linked region in an XY system, the lower number of functional copies of sex-linked genes in males than females may lead to disadvantageously low expression of many genes, and natural selection should then favour dosage compensation to restore the diploid level. However, it is important to test, not only whether dosage compensation occurs in a species that has evolved a sex-linked region, but also whether it evolved in response to genetic degeneration. The alternative is the existence of cellular homeostasis systems that can up-regulate expression for a gene that is heterozygous for a mutant or deleted copy (reviewed by Malone *et al.*, 2012; Birchler, 2013).

Although the time-course of gene losses from young Y chromosomes, and deterioration of function, are rarely known, and probably differ greatly in systems with different sized non-recombining regions, these processes occur gradually, so that compensation might initially evolve independently for each gene affected. Moreover, chromosome-wide dosage compensation is likely to be disadvantageous in young sex chromosomes, as this would result in males over-expressing any genes still present on the Y, like a triploid aneuploid for this chromosome. Furthermore, increased expression is unlikely initially to be restricted to

575 males, but might evolve if the advantage of correcting low male expression outweighs this
576 disadvantage, adding a further disadvantage of over-expression in females (Charlesworth,
577 1996). In *Silene latifolia*, a special form of imprinting causing increased expression from the
578 maternal X chromosome in both sexes, compared with autosomal genes, has been
579 proposed; this would avoid overexpression in males, but not in females (Muyle *et al.*, 2018).
580 This species, and other plants, are likely to be excellent systems for evaluating the time
581 course of evolution of dosage compensation in sex chromosomes, and whether the relative
582 ages of sex chromosome systems can be inferred from the existence, or not, of a dosage
583 compensation system, and whether individual genes show compensation, or a
584 chromosome-level system has evolved (see box). Currently, expression estimates remain
585 difficult and errors of the expression are high, as exemplified by differing conclusions about
586 dosage compensation for the same species, such as the old (degenerated) stratum of the
587 threespine stickleback (Schultheiß *et al.*, 2015; White *et al.*, 2015). Clearly, more work is
588 needed in the future, to understand many of the puzzles outlined here.

589

590 **Acknowledgements**: I am grateful to the ERC for funding my research by an ERC Advanced
591 grant, ERC grant number 695225 (GUPPYSEX).

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893 **Figure legends**

894

895 **Figure 1.** Two possible ways in which separate sexes might arise in related species, with
896 changes in the sex system shown as dotted lines in the tree. **A** shows the case when dioecy
897 evolved in an ancestor of three species, one of which has reverted to functional
898 hermaphroditism. The phylogeny could potentially reveal the evolutionary events, provided
899 that a fully sex-linked sequence in the oldest part of the sex chromosome pair can be
900 identified: for example, in an XY system, the divergence between the alleles in either
901 dioecious species will then date back to the dioecious common ancestor, and should exceed
902 divergence of either species from the hermaphrodite species. It may be possible to infer
903 whether the hermaphrodite evolved by a change in the Y or the X. For example, papaya
904 hermaphrodites carry a Y chromosome that probably arose by deletion of a Y-linked female
905 suppressor (Wang *et al.*, 2012). A related species, *Vasconcellea monoica*, has reverted to
906 functional hermaphroditism, and orthologues of papaya sex-linked genes have almost
907 identical divergence from papaya X and Y alleles.; loss of dioecy therefore occurred before,
908 or soon after, these genes stopped recombining in papaya (Gschwend *et al.*, 2011). **B** shows
909 a case where the evolutionary history is less clear. Dioecy could have evolved independently
910 in the two dioecious species at different times, so that XY sequence divergence differ in the
911 two, reflecting the different times. However, similar divergence differences can arise if the
912 true evolutionary history is the one shown in part A, but the sequences being compared are
913 in a younger evolutionary stratum and started diverging recently. If the hermaphrodite is
914 extinct, one might conclude that dioecy is the ancestral state, unless X-Y divergence is
915 known

916

917 **Figure 2.** Diagram to illustrate how a non-recombining region may evolve in a chromosome
918 carrying a sex-determining locus, and later disappear, for the case of male heterogamety.
919 Chromosomes are shown as horizontal lines. At the top, an ancestral chromosome with a
920 single sex-determining gene is assumed. Unless the presence of the gene directly causes
921 suppressed recombination (see Figure 3), sex linkage will be confined to this gene, and the
922 chromosome pair will be homomorphic (only the "Y" is shown, as its homologue will be the
923 same, apart from the maleness factor or male-sterility mutation, either a mutation in a
924 coding sequence of a gene essential for male function, or a change preventing expression of

925 such a gene). If a sexually antagonistic (SA) mutation occurs in the region and establishes a
926 polymorphism, then, because the mutation is disadvantageous in females, this generates
927 selection for close linkage with the sex-determining gene, or male-specific expression of the
928 male-benefit trait (Outcomes 1 and 2, respectively). An extensive Y-linked region may
929 evolve, including both these gene and many others in the region (wide blue bar), creating
930 true X- and Y-linked regions. An inversion might cause suppressed recombination, directly
931 producing morphologically different X and Y chromosomes, or heteromorphism may evolve
932 later, as well as genetic degeneration. The bottom diagram shows a possible later stage,
933 when the male-determining factor is replaced by a new one taking control of sex-
934 determination; sex-determination is then again under single gene control and the sex
935 chromosome pair is again homomorphic.
936
937 **Figure 3.** An evolutionary scenario incorporating the findings in the persimmon (see
938 explanations in the main text). Three different chromosomes are symbolised by horizontal
939 pink, green and black lines, and the genes in the model are coloured to match their
940 chromosomes, except that the maleness factor on the black chromosome is in blue. The
941 diagram shows the effects of the two mutations involved in the evolution of females (first)
942 and then males; each of the mutations acts by affecting expression of a different gene. Note
943 the insertion that created the female suppressor, and the effect of the insertion in creating
944 a region of non-pairing in this chromosome region (grey), and potentially in the region
945 flanking the insertion, so that recombination is suppressed creating XY-linked regions (red).

Box: The stages in sex chromosome evolution, illustrated for the case of an XY system

The stages illustrate (i) that the age of a sex chromosome system is best understood in relation to the different kinds of events and changes from the ancestral state that can, but need not inevitably, occur, and (ii) the potentiality for a lineage with an old system to change back to a new and younger state. Ages are not given in terms of years, for two reasons. First, the number of generations, not years, is most relevant to the stage that will be reached, including the extent of sequence divergence between Y- and X-linked sequences and of genetic degeneration. Second, such processes occur at different rates per generation, depending on factors including the species' effective population size (Bachtrog, 2008). However, some estimates in years are mentioned, to provide rough information; these values illustrate that a later stage can be found in a species at an earlier stage, as there is no necessary progression from stages 1 to 2 and onwards. For example, the persimmon case is listed among young systems, because a single-gene system will be young when it first evolves, and may not lead to the establishment of diverged X- and Y-linked regions, even if it is maintained for a long evolutionary time.

Stage	Evolutionary event	Possible consequences of the event	Examples (plant examples in bold)	References
1: early stage of young systems	Evolution of a sex-determining gene or genes on a chromosome that was not formerly a sex chromosome	In a single-gene system (see below, and the main text, for situations in which such systems can arise), the sex-determining region may retain crossing over	Fugu and possibly guppy **Persimmon, kiwifruit (**20 MYA[1])	(Kamiya *et al.*, 2012), (Bergero *et al.*, 2019) (Akagi *et al.*, 2014), (Akagi *et al.*, 2018)
2	Evolution of suppressed recombination: if two sex-determining genes are involved, closer linkage may evolve between them, creating a young "proto-Y and X" sex chromosome system [2]	a) Sexually antagonistic polymorphism(s) may become established closely linked to a sex-determining locus, promoting evolution of even less recombination in the region b) The genome region in which recombination is suppressed may be a small part of the chromosome, leaving most of the chromosome as a pseudo-autosomal region	**Papaya** (7 MY) **Asparagus (2 MY)** [3] Several fish, including cichlids	(Liu *et al.*, 2004) Harkess et al., 2017 (Gammerdinger & Kocher, 2018b)

[1] Age estimated from divergence of the male-determining factor and the gene from which it appears to have evolved by duplication

[2] Multiple events suppressing Y-X recombination may occur at different times, creating "evolutionary strata" of Y-X divergence (see main text)

[3] **Norup M, Petersen G, Burrows S, Bouchenak-Khelladi Y, Leebens-Mack J, Pires J, Linder H, Seberg O. 2015.** Evolution of *Asparagus* L. (Asparagaceae): Out-of-South-Africa and multiple origins of sexual dimorphism. *Molecular Phylogenetics and Evolution* **92**: 25–44.5

		c) The non-recombining region may extend beyond the region containing the two genes		
3	Divergence between Y-linked and X-linked homologues	Repetitive sequences accumulate and X-Y differences develop (heteromorphism and low gene density) [4]	**Papaya, *Silene latifolia*** (6-10 MY) Threespine stickleback, guppy	(Wang *et al.*, 2012), (Gschwend *et al.*, 2011) (White *et al.*, 2015), (Nanda *et al.*, 2014)
4	Y-linked genes' functions may degenerate	Closely linked transposable element sequences may affect gene expression	***Rumex rothschildianus, Silene latifolia*** Threespine stickleback	Stephen Wright's article in this volume, (Bachtrog, 2008) (Papadopulos *et al.*, 2015) (White *et al.*, 2015)
5: ancient systems	Dosage compensation of genes in non-recombining regions may evolve to restore adequate male gene expression levels (of X-linked copies)	Dosage compensation may act on expression of individual genes, and eventually at the whole chromosome level	Drosophila, mammals (>150 MY), birds ***Silene latifolia***	Reviewed by (Vicoso & Bachtrog, 2009), Bachtrog, 2008 (Muyle *et al.*, 2012)
6	Y-linked regions lacking functional copies of essential genes may be deleted, leading to hemizygosity in males	a) Possible evolution of a new male-determining gene on a non-Y chromosome, either an autosome or the X, or movement of an existing male-determining gene to a new location on the X or an autosome, creating a single gene sex-determining system, returning the species to "young" state 1 above b) Possible loss of essential male function genes from the Y, creating an X0 system	***Marchantia polymorpha***	(Ohyama *et al.*, 2001) reviewed in (Beukeboom & Perrin, 2014)

[4] For example, an inversion may suppress crossovers between them and also across a larger genome region, or crossovers may be suppressed in meiosis of one sex, potentially in the entire genome, as in Dipteran males